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OBSERVATIONS ON THE SEXUAL BEHAVIOR OF FREE-FLYING *AEDES AEGYPTI* MOSQUITOES[1]

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The first description of copulation of mosquitoes was given by Godeheu de Riville (1760). At the present time the most detailed observations on sexual behavior in mosquitoes are those of Roth (1948), Spielman (1964), and Jones (1973), who worked primarily with free-flying *Aedes aegypti,* and those of Jones and Wheeler (1965) who used mostly the forced-copulation technique. Roth (1948, page 270) concluded that *A. aegypti* mosquitoes ". . . copulate most frequently after the female comes to rest, while copulation partly in flight and at rest is common and copulation exclusively in flight is the least common." He never observed copulation when the male was "astride" the female.

Since Godeheu de Riville (1760), it has been known that *A. aegypti* copulates belly-to-belly or face-to-face, and that the angle formed between the copulating pair varies from 45° to 90°. Once the male is in position he uses mainly his front tarsi to hold the female's hind femora or her other legs. He is also said to use his metathoracic legs to push the female's abdomen or her hind legs just prior to genital contact. After terminalial contact, the male's mesothoracic legs may push on the female's tibiae or tarsi to keep them raised during coitus. Sometimes the claws of the male's metathoracic legs are said to be hooked over the margins of the female's wings (Roth, 1948).

Roth's work (1948) gives the strong impression that copulation occurs almost reflexly, whenever flight is induced. Thus, he states ". . . disturbance induces them to fly and the males immediately pursue the females and seize them" (page 272), and he further wrote that males copulate ". . . at any time of the day, until death, whenever stimulated by flying females" (page 346).

Materials and Methods

All of the observations were made using adults of either the Bangkok or the U. S. Naval Medical strains of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus). The behavior of the mosquitoes was examined while they were confined in either 600 ml or 1000 ml glass beakers with mosquito netting tops or within 1 cu ft cages having one or more clear plastic sides. The insectary was held at 27° ± 2° C and 80% ± 2% R.H. The constant air circulation within the insectary was associated with a rather loud continuous background noise. The insectary was illuminated from 0630 to 1900 hours daily. The observations were made only under well-lighted conditions between 0800 and 1800 hours.

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Observations

Recognition and chasing

Resting males as a rule are not activated to flight by the mere presence of flying females in a 1 cu ft cage. Further, resting males often remain stationary even when females fly within 2 inches of them. In colony cages, males often remain standing despite flights closer than one inch by females, providing a female does not touch him with her trailing legs. Often a female skimming across the surface of a wall may contact a male with her legs and he is then induced to fly. In colony cages, we have seen stationary males which did not fly when females landed very close to them or even when such females happened to touch the males so that the pair were in tarsal contact. In cages containing only one male, if the females come within about 1 inch or less of him, he almost always takes flight very quickly.

If a male is already in flight, the presence of one or many flying females does not necessarily elicit any directed response by a given male. The presence of several males in a cage (some of which may be flying at any given moment) appears to increase the extent of flight by the males as a group so that they can chase and capture more females than solitary males. Males which have been flying in a group for some time seem to be less alert in locating females than those previously stationary males which have just been jostled or otherwise induced to fly either by another male or a female landing on their backs in a colony cage. Females have been seen to fly around or close by stationary or flying males or to cross flight paths with a male and still not be pursued. This was especially noticed with seminally depleted males, but could also be observed even with 3 to 4 day old virgin males which had rotated their terminalia normally and which had extended hairs on their antennae.

Often a flying male was seen to change a presumably undirected flight pattern suddenly, and then rapidly chase a flying female. A pursuing male can cease chasing a given female for no discernible reason and may continue to fly or else land. Occasionally we have observed females whose power of flight and/or maneuverability considerably exceeded those of the males, so that they were able repeatedly to elude any chasing individual from a group. While a very few females successfully avoid capture for extended periods, eventually most of these will be caught. Females can also avoid capture by frequently landing (being air-borne for only a short period at a time), by standing for long periods, or by skimming along the ceiling, floor, or walls at varying heights. Males frequently capture females without a conspicuous or prolonged chase; but, if they do so, males may suddenly fly much faster than normal when chasing some females and often appear to make a series of short zigzags as they get near to one of them.

Capture

During the pursuit the male may fly in one or more loops around a given female. After such a look-over, a male may either attempt to make tarsal contact with a flying female or may cease the chase and generally quickly land or may begin to pursue another female. When the mating status of a female was known, it was observed that males could readily chase, capture, and assume a copulatory

position with both virgin and previously mated females. Further, the duration of genital contacts were within the same range for both types of female.

Once a male has captured a female, grasping her with his prothoracic and perhaps mesothoracic legs, he generally quickly orients himself so that he is ventral to her. Prior to genital contact in flight, the male's abdomen generally hangs slightly downward, parallel to that of the female. At this moment, a number of possible actions may occur, depending upon the activities of the male or female partner. A broad category of distinction may be drawn between activities which occur if the female remains flying with the attached male or if the pair land.

Mid-air copulations

If a female continues to fly with the male holding on in a ventral position, the male may arch his abdomen towards the female and attempt to clasp her cerci. If cercal contact occurs, their flight suddenly and recognizably changes so that the previous rapid spiral and zigzag motions of the pair slow down perceptibly to become smooth, circular or spiral patterns. During this time of slowed flight, it can often be seen that the male's wings are not beating and it can also be determined that the male is indeed in firm genital contact with the female.

If genital union has been made by a flying couple, copulations of variable duration may occur exclusively while air-borne. Because the individuals are moving so rapidly, sometimes in the midst of others, it is frequently not possible to record air-borne coital time with high accuracy. Termination of in-flight coitus can be noted by the sudden lowering of the male's abdomen, and this action is generally rapidly followed by the departure of the flying male. Some air-borne couples which have already achieved firm genital union may land with the female in a position above the male without coitus being interrupted. While most couples land on a side of the cage, some may land on the floor.

Many copulations begin during flight and are broken up on landing. This separation appears to depend to a large degree on the stability of the landing pair and the character of the substrate. Thus, if a copulating couple land on a cloth sleeve or mosquito netting, their union tends to remain firm, but if a couple land on the metal floor or on the edge of the cage, the genital contacts are often broken. For example, a female may land on a ledge so that the male is suddenly violently struck on the head or pronotum by the ledge at the moment of landing. Sometimes a joined couple may land apparently off-balance and before the female can come to a complete halt, the male will have been abruptly dislodged on impact with the substrate. A number of mid-air copulations of long duration (which were apparently nearing their end) were terminated almost immediately, but some couples continue to copulate for a short time after landing.

Where numerous males are present in a cage, the copulation of an individual couple may be broken up in mid-air by another male attempting to seize the same female. Occasionally a pair which may or may not have achieved genital union, while still air-borne, may attract more than one flying male and this may result either in the rapid separation of the entire group or else the intruding males will make tarsal contacts with the pair, causing highly irregular and rapid flight. Most often, due to the added weight, the group will land on the floor. The female and

her accompanying males may then exhibit tumbling, as the males vie to achieve cercal contact. This tumbling may result in cercal clasping by one male while the others remain tarsally in contact with the copulating pair. The other males may become catatonic or make repeated abdominal archings towards the joined pair. Sometimes, these attempts to clasp by intruders will dislodge the first copulating male, resulting either in departure in flight by the female or successful genital contact by one of the intruders. Sometimes after a female has departed, a pair of males may be seen on the floor, flexing their abdomens and attempting to clasp each other.

Copulation following landing

In most cases couples land before the male has made terminalial contact. If the female lands while the male is still attempting to clasp her cerci, the couple will stay tarsally united. The success of cercal clasping depends greatly on the final position of the couple after landing. Thus, a male which has maintained coital alignment will be far enough forward relative to the body of the female so that when he arches his abdomen, the terminalia of the two can meet. Often during landing the male may end up too far posteriorly relative to the female, and thus cannot attain genital contact by abdominal flexing. The male may, nonetheless, continue to make clasping movements. Slightly misaligned males may later gain a more favorable coital position and succeed in making a firm genital contact.

During cercal clasping and copulation in a standing position, the hind legs of the female are generally raised unless the couple's position is unstable, necessitating further bracing by the female's lowered metathoracic legs. When the hind legs of the female are elevated, this may be due either to her own action or to the male's pushing against them with either his mesothoracic or metathoracic legs, with the result that the female's legs are spread out of the way. We have never seen the claws of the male's metathoracic legs hooked over the female's wings. The male's metathoracic legs may be extended from his body against the substrate to form a stabilizing base from which abdominal arching and copulation can occur. The actual clasping of a female's terminalium and subsequent copulation generally result in a slight downward pull of the tip of the female's abdomen by the male. We have seen the hind legs of some seminally depleted males quivering towards the end of a non-inseminating copulation. On many occasions, the slow lowering of the female's hind legs, on her own or due to their release by the supporting legs of the male, occurs just prior to the breaking of genital union and can be a clue to an impending termination of copulation. On a few occasions, in attempting to leave the female after a long copulation, a male may become twisted into a position 180° from that of the female and remain briefly terminally joined to her. A further struggle generally breaks the connection. Shortly after a couple concludes copulation, a male may quickly copulate again with the same female. This subsequent copulation is usually significantly shorter than the first. Very rarely, the female may fly off with a terminally-attached male and the two separate in flight.

Some couples were occasionally observed to land on the mosquito netting on the roof of the cage and these generally then copulated, the pair being suspended only by the prothoracic leg or legs of the female. The duration of such copulations were no different from seemingly more stable positions. Just prior to breaking

genital contact, the male releases his tarsal hold on the female and may swing downward before departing. On several occasions, genital contact was not broken at this time so that the male continued his downward swing through a wide arc, often as much as 180° before the union was severed. Occasionally a couple flew off together from the roof, sometimes still *in copula*, but more often after termination of copulation, so that the partners separated from each other in flight.

Sometimes when couples land on the floor, the struggling pair exhibit violent tumbling and kicking, especially if a firm genital contact had not been achieved in mid-air or had been broken upon landing. On quite rare occasions the male of a couple that has not yet attained a copulatory position in flight may end up in a position superior to the female upon landing. He may be able to clasp her cerci momentarily before being dislodged by her struggles. On only one occasion was the duration of the terminalial union barely sufficient to be classified as a possible copulation (he did not inseminate her).

Males have been seen to chase and capture females and although seemingly perfectly aligned in a normal copulatory pose only lightly touch their terminalia to those of the females once or many times in rapid succession. They sometimes succeed in lightly clasping the cerci or terminal sterna of the females but do not establish firm genital unions. Seminally depleted males reach a stage when they will chase and capture females but no longer copulate with them. Such males were often seen to be correctly aligned and often made repeated light terminalial touches.

Grossly misaligned males

A grossly misaligned male may continue flexing his abdomen even though his terminalium does not contact the body of a female. Such males may be diagonally or laterally positioned so that only minimal tarsal contact with a female is maintained at this time. Repeated kicking and clasping attempts by such males may position his head under the female's terminalium.

A female which is being chased by a male may quickly land and the male may as suddenly land very close to or on the back of the female. Generally, as soon as the male contacts the body of the female, she rapidly takes flight and is then pursued again by the male and is often captured. If the female remains stationary, however, with the male on her back, several things can happen. Males which have landed on the backs of females are quite active and appear to walk over the female but do not attempt to clasp and never achieve genital contact of any kind from a location on top of her back. Males which have landed close beside a recently landed female may begin to flex their abdomen toward the female but are so misaligned that firm genital contact cannot be made. Often during this activity the female may kick the male with one or both metathoracic legs and thus drive him away. It should be noted here that when a female lands upon the back of another female or otherwise comes in contact with one, both females tend to remain standing quietly without kicking. When a normal male fails to make cercal contact due to his being severely misaligned, it is the female which flies away, as he tries to clasp either her thorax or abdomen with his claspers. Nevertheless, many females have been seen to remain standing despite being vigorously and frequently

jostled by misaligned males. An exception to this behavior is that seminally depleted males are nearly always the ones to leave the female. Such males were often misaligned and they generally flew away after a brief attempt to become realigned. Even when the depleted male is being carried by the flying female, he may suddenly lose tarsal contact and drop abruptly downward. On one unequivocal occasion a male landed on the side of the cage close beside a long-standing female (with raised metathoracic legs) and, passing in from the side, was seen to position himself very rapidly underneath her and succeeded in clasping her terminalium and copulated.

Successful copulatory positions

The positions of the male relative to the female that may result in firm genital unions vary quite remarkably. For example, a male may copulate when he is located slightly, and very rarely far, to one side of the female or when he is positioned so far to the rear of her that he is balanced on the front of his pronotum with the result that his abdomen must be strongly upcurved to achieve genital union with a coital angle approaching 90°. A rarely seen variation on this position occurs when a female attracts 2 males simultaneously: upon landing she appears to fall forward which elevates her terminalium so that firm genital contact by one of the males and clasping attempts by the other result in a tripod-like configuration.

If the initial clasping attempts of the male are not successful, both partners may become motionless for variable (often for quite long) periods. It should be noted that during such catatonic states the male remains in firm tarsal contact with the female. Clasping attempts may then resume which may or may not result in cercal contact, depending upon the male's alignment. At no time during this clasping activity (of either constantly active males or those resuming after a catatonic period) was the female ever observed to change her stance to facilitate terminalial union (nor was the male ever observed to elevate the female's abdomen to gain a more favorable coital position). Sometimes when a couple had not achieved terminalial union after several or many clasping attempts by the male, one or both partners (but generally the female) would terminate the activities by flight, leaving the other partner. Sometimes males would hang onto the female and be carried by her to another location where copulatory acts might be resumed.

Termination of copulation

As previously noted, the termination of copulation is often signalled when the female's metathoracic legs descend. The instant of the breaking of firm genital unions is marked by a sudden springing apart of their terminalia. If genital separation does not occur during lowering of the female's hind legs or immediately thereafter, she usually quickly raises them again and brings them abruptly down towards the male, thus kicking his abdomen or at his terminalium. This action may have to be repeated several times before the male will depart. Another technique which may be used separately or in conjunction with the above is the crossing of her hind legs during lowering so that they are placed in the space

between their abdomens. Extension of her crossed legs strikes the joined terminalia and usually without fail leads to his separation. After copulation for 6 secs. or longer, a female may suddenly lift her body upon her greatly extended legs and this action alone may dislodge some males. Sometimes a female may use all or any combination of the above techniques to break genital union. Soon after the female is alone, either having flown off by herself or after the male has left, she may be seen to scrub her metathoracic legs, often rubbing them over her terminalium. However, the occurrence of this action may not necessarily indicate a recently copulated female. We have seen seminally depleted males repeatedly scrubbing their terminalia in a similar manner.

Discussion

Contrary to a widely-held belief gained from Roth (1948), males are not instantly or regularly induced to fly and chase flying females under uncrowded conditions. In fact, most of a male's time is spent either in standing or in apparently non-directed flights. It is only when colony cages containing large numbers of mosquitoes of both sexes are observed that one obtains the impression that sexual behavior is occurring relatively constantly. However, close observations on individual males even in such cases will reveal that only during a small percentage of a male's time is he actually chasing a female or engaging in copulation.

While it can be said that males locate flying females by means of their antennae which are acting to discern the sounds of a female's wing beats (Roth, 1948), the present work indicates that this sensory mechanism of the male may not always activate him to flight or else may not itself be activated by the sound of the female wingbeats. Since males do not always respond to the sound of a flying female, it might be that the response to imputs from this mechanism occurs at will or else is triggered at varying thresholds. Roth (1948) concluded that an adaptation occurred among males which had been exposed for varying periods to sounds simulating female wingbeat frequencies. It may be that the periods during which a male does not respond to the sound of a flying female may represent periods of adaptation to the general sound of females flying in a cage. Activation to flight may then be interpreted as a response to a sound of slightly different but distinguishable frequency from the background noise to which he has adapted, or else to a sound that exceeds, due to the proximity of the female, the level of adaptation.

The performance of an average male in detecting and capturing a female seems truly remarkable considering the inherent difficulties of locating rapidly flying females which are constantly changing flight directions due to the confines of the cage. Furthermore, this ability to distinguish the flight tone of a female as opposed to sound reflexions from either curved glass or smooth plastic surfaces seems all the more striking. Where numerous females are flying in a cage, the ability of a male to locate and track a single female in the midst of interfering noises from other flying females (even allowing for slight variations in flight tone due to age or weight) would indicate truly formidable powers.

It has not been previously noted before this that sometimes despite vigorous chases by males, some females can elude capture for long periods by various tactics.

Once a male has captured a female and begins to copulate with her, the coital angles are essentially the same irrespective of the site of copulation. In agreement with Roth (1948), the present observations indicate that copulations most frequently occur on the landing of a pair which had previously been partially joined in flight. The second most common site of copulation is partially in flight with termination shortly after landing. Less frequently, copulations occur entirely in the air and are terminated while the pair are still in flight. The least common method of copulation was the one case where a male succeeded in aligning himself under a long-standing female and copulating with her.

The effects of changing flight tone to one which is presumably more noticeable to sound-adapted males is demonstrated by the attraction of other males to a couple which have just initiated a steady slow flight pattern.

The importance of the male gaining a firm hold on the female in a position from which his terminalium can touch that of the female prior to her landing is shown by the relative lack of success of misaligned males to achieve copulation.

Adult males will not copulate with freshly inseminated females or with those which have been inseminated for a long time if their terminalia are forcibly rubbed together under a microscope (Jones and Wheeler, 1965). The males make no attempt or only feeble attempts to clasp the cerci of females which are manually presented to them. Furthermore, the males under such artificial conditions will not attempt to grasp the cerci even when the terminal segments of the female are distended so that the cerci are readily available (Jones, unpublished). It is very evident from the present study however that this phenomenon does *not* occur with free-flying mosquitoes. Free-flying males apparently cannot recognize virgin from inseminated females since they readily clasped and maintained a copulatory position with both, usually for a similar period of time.

Termination of copulation may be due to actions of either the male or the female. The female certainly appears to terminate most genital unions by actively dislodging the male, primarily by striking him with her metathoracic legs or by standing on her tarsal tips. It is possible that she cues the male to withdraw and that the lowering of her hind legs is sometimes due to his activities. In order to terminate copulation, the male must reverse the copulatory acts, as follows. He must withdraw his aedeagus from the vagina, then retract his apical paraprocts from her cloacal hollow, and finally must release her cerci.

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Summary

1. The sexual behavior of 2 strains of free-flying *Aedes aegypti* in 1 cu ft cages is described.

2. Groups of resting males are not necessarily activated to flight by flying females under uncrowded conditions, even though the females may fly within 2

inches of them. Individual males tend to be activated to flight if females fly within one inch of them or actually touch them.

3. Flying females do not necessarily elicit directed responses by flying males. When males pursue females, they may fly in one or more loops around them before establishing tarsal contact. Only a small percentage of a male's time is spent in chasing or copulating with females.

4. Copulations taking place in mid-air can be recognized by a sudden change in the flight pattern of the couple from a rapid zigzag to a slower spiralling path. Most couples land before the male makes genital contact with the female. On many occasions males were seen to land beside females and to be in tarsal contact with them. Although some of these males made attempts to clasp stationary females from the side, on only one occasion was a male actually seen to crawl beneath a long-standing female and to copulate with her.

5. Females were never seen to change their stance to facilitate copulatory acts of the males. Although males and/or females may terminate copulation, the lowering of the female's metathoracic legs generally signals an impending end to the act. Females may additionally have to cross their hind legs, stand on their tarsal tips or fly away to dislodge some males.

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